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TRANSACTIONS

Über die Bestandteile des Zuckerrohrs.

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Das Zuckerrohr kann neben der Zuckerproduktion auch für die Herstellung von Alkohol, Zellstoff, Furfurol, Glycerin, Aceton usw. benutzt werden. Eingehende Angaben über die physikalischen Eigenschaften sowie die chemischen Bestandteile des Zuckerrohrstengels sind aber sehr spärlich. In der vorliegenden Arbeit haben wir einige im hiesigen Institut gezüchtete vorzügliche Varietäten von *Saccharum officinarum* als Versuchsmaterialien gewählt und zunächst morphologische sowie chemische Untersuchungen ausgeführt. Dann wurde die Herstellung des Zellstoffs aus der Bagasse versucht.

1. MORPHOLOGISCHE UNTERSUCHUNGEN DES ZUCKERROHRSTENGELS.

Das 18 Monate alte Zuckerrohr wurde gepreßt, die erhaltene Bagasse mit Schulzischer Lösung behandelt und die Form der Bagassezelle mikroskopisch beobachtet. Auf Grund dieser Beobachtung haben wir die Bagassezellen in drei Gruppen geteilt und die Zahl der zu jeder Gruppe gehörigen Zellen bestimmt (Tabelle I).

Tabelle I.

Varietät des Zuckerrohrs	F 108	F 109	F 110	F 111	Mittel
Faserzelle in %	42,90	54,08	47,79	35,05	44,66
Rechteckige Zelle in %	29,31	18,96	24,45	34,17	27,02
Weiche Zelle in %	27,39	26,96	27,76	30,78	28,32

Ferner wurden die durchschnittliche Länge und Breite dieser Zellen ermittelt (Tabelle II).

Tabelle II.

		F 108	F 109	F 110	F 111
Faserzelle	Länge in mm	1,172	1,051	1,138	1,155
	Breite in mm	0,018	0,018	0,018	0,018
Rechteckige Zelle	Länge in mm	0,305	0,287	0,325	0,343
	Breite in mm	0,053	0,066	0,070	0,068
Weiche Zelle	Länge in mm	0,231	0,205	0,221	0,268
	Breite in mm	0,147	0,133	0,130	0,140

Die Resultate der genaueren Bestimmungen enthalten die Tabellen III, IV und V.

Tabelle III.

Länge der Faser.

Länge in mm	F 108	F 109	F 110	F 111
	In % der gesamten Zellen			
0, — 0,25	0	0	0	0
0,25 — 0,50	6,87	6,30	9,31	6,91
0,50 — 0,75	24,89	23,40	23,08	18,43
0,75 — 1,00	19,31	24,47	19,84	26,27
1,00 — 1,25	10,73	19,50	9,72	13,82
1,25 — 1,50	13,73	8,87	11,34	12,90
1,50 — 1,75	9,01	8,16	8,91	7,37
1,75 — 2,00	5,15	4,96	8,50	6,45
2,00 — 2,25	2,58	2,13	3,64	3,23
2,25 — 2,50	3,43	1,42	4,80	0,46
2,50 — 2,70	1,29	1,06	0	1,84
2,75 — 3,00	2,58	0	0	0,46
3,00 — 3,25	0	0	0,41	1,38
3,25 — 3,50	0,43	0	0	0
3,50 — 3,75	0	0	0,41	0,46

Tabelle IV.

Breite der Faser.

Breite in mm	F 108	F 109	F 110	F 111
	In % der gesamten Zellen			
0, — 0,01	6,8	6,5	6,5	6,0
0,01 — 0,0125	22,2	10,5	14,5	15,5
0,0125 — 0,0150	13,0	12,0	13,5	14,5
0,0150 — 0,0175	14,0	12,5	17,0	12,5
0,0175 — 0,0200	9,2	19,5	15,5	16,5
0,0200 — 0,0225	18,4	18,0	13,5	15,5
0,0225 — 0,0250	4,8	7,0	8,0	8,0

0,0250 — 0,0275	2,9	3,0	3,0	2,0
0,0275 — 0,0300	1,5	3,0	1,5	3,0
0,0300 — 0,0325	3,4	4,0	4,5	3,0
0,0325 — 0,0350	1,5	1,0	1,0	0,5
0,0350 — 0,0375	0	0,5	1,0	0,5
0,0375 — 0,0400	0,5	1,0	0,5	0
0,0400 — 0,0425	0	1,0	0	2,5
0,0425 — 0,0450	1,5	1,0	0	0
0,0450 — 0,0475	0,5	0,5	0	0

Tabelle V.
Länge der rechteckigen Zelle.

Länge in mm	F 108	F 109	F 110	F 111
	In % der gesamten Zellen			
0 — 0,1	0,43	0,64	0,30	0,25
0,1 — 0,2	21,74	18,59	11,62	11,00
0,2 — 0,3	40,00	47,50	39,38	36,33
0,3 — 0,4	23,91	22,44	31,61	29,90
0,4 — 0,5	9,13	5,77	10,36	14,47
0,5 — 0,6	1,74	1,92	3,63	5,47
0,6 — 0,7	1,74	1,20	1,55	5,47
0,7 — 0,8	0,87	0,80	1,80	1,93
0,8 — 0,9	0,40	0,50	0,24	0,64
0,9 — 1,0	0,03	0,64	0,32	0

2. CHEMISCHE UNTERSUCHUNGEN DES ZUCKERROHRSTENGELS.

Zunächst wurden das spezifische Gewicht und der Zuckergehalt des aus dem 18 Monate alten Zuckerrohr gewonnenen Saftes ermittelt (Tabelle VI).

Tabelle VI.

	F 108	F 109	F 110	F 111
Grade Brix	21,9	19,7	19,8	21,3
Grade Pol	20,1	18,9	18,8	20,2

Die Bagasse wurde nach der vollständigen Entfernung des Zuckers auf übliche Weise analysiert (Tabelle VII u. VIII).

Tabelle VII.

	F 108	F 109	F 110	F 111
	In % der Trockensubstanz			
Asche	1,49	1,39	1,55	1,64
Kalt-Wasser-Extrakt	2,54	1,94	2,32	2,24

Heiß-Wasser-Extrakt	4,48	4,49	4,57	3,96
Verd. Alkali-Extrakt	36,95	37,30	33,90	38,80
Alkohol-Benzol-Extrakt	2,76	2,83	2,70	2,69
Pentosan	27,02	27,23	27,53	26,19
Lignin	21,81	20,10	21,64	18,63
Stickstoff	0,34	0,33	0,35	0,35
Cellulose	50,32	47,75	49,18	47,93
α -Cellulose	37,23	34,85	35,10	34,72
β -Cellulose	6,43	7,12	6,02	4,97
γ -Cellulose	6,64	5,79	8,05	8,22

Tabelle VIII.

	In % des frischen Gewichts des Zuckerrohrstengels			
Zucker	16,28	15,79	15,69	16,58
Heiß-Wasser-Extrakt	12,91	12,01	12,23	11,64
Alkohol-Benzol-Extrakt	0,37	0,36	0,35	0,33
Pentosan	3,65	3,42	3,53	3,18
Lignin	2,92	2,53	2,77	2,26
Cellulose	6,80	6,01	6,30	5,81
α -Cellulose	5,04	4,38	4,50	4,21
β -Cellulose	0,87	0,89	0,77	0,60
γ -Cellulose	0,90	0,73	1,03	0,99

3. VERSUCHE ZUR HERSTELLUNG DES ZELLSTOFFS AUS DEM ZUCKERROHRSTENGEL.

Der Zuckerrohrstengel wurde gepreßt und die zurückgebliebene Bagasse nach der Entfernung des Zuckers mit Wasser pulverisiert. Die Analyse der Bagasse gibt das folgende Resultat (Tabelle IX).

Tabelle IX.

Wasser	8,94%	Cellulose	49,97%	Asche	2,03%
Heiß-Wasser-Extrakt	1,66	In % der Cellulose	α -Cellulose	Lignin	22,08
Verd. Alkali-Extrakt	29,70		β -Cellulose	Pentosan	27,81
Alkohol-Benzol-Extrakt	3,37		γ -Cellulose		

100 g Bagasse wurden mit 42 g Natriumhydroxyd und 700 ccm Wasser versetzt und dann innerhalb einer Stunde bis zu 165° erhitzt. Nach bestimmten Zeiten wurde mit der Erhitzung aufgehört und die aufgeschlossene Substanz innerhalb 2½ Stunden bis zur Zimmertemperatur erkalteten gelassen. Die Analyse der gewonnenen Zellstoffe ergab folgendes (Tabelle X).

Tabelle X.

Dauer der max. Temp. in Std.	$\frac{1}{2}$	1	2	3	5
Ausbeute in %	36,52	35,14	33,36	30,97	29,12
	In % des Zellstoffs				
Pentosan	12,72	11,53	11,45	11,05	11,02
Lignin	3,82	3,09	2,92	2,70	2,02
Asche	2,11	1,41	1,42	1,55	1,17
Cellulose	92,57	92,77	93,53	95,91	97,41
In % der Cellulose					
α -Cellulose	85,30	82,75	78,75	74,78	76,41
β -Cellulose	11,44	13,96	16,05	22,60	20,22
γ -Cellulose	3,27	3,29	5,20	2,62	3,37

Der Soda- und Zuckergehalt der nach dem Aufschluß erhaltenen Lösung sind in Tabelle XI verzeichnet.

Tabelle XI.

Dauer der max. Temp. in Std.	$\frac{1}{2}$	1	2	3	5
NaOH in %	22,2	20,0	17,9	15,2	12,4
Na ₂ CO ₃ in %	3,2	4,0	4,2	6,4	7,2
Reduzierender Zucker in %	1,84	1,18	1,00	1,59	2,16
Gesamtzucker in %	4,78	3,92	4,05	4,37	4,60

Wir haben dann die Bagasse vor dem Aufschluß mit heißem Wasser vorbehandelt. Zu diesem Zwecke wurden 100 g Bagasse mit 500 cm Wasser gekocht. Die chemische Zusammensetzung der so vorbehandelten Bagasse ist die folgende (Tabelle XII).

Tabelle XII.

Max. Druck in Pfunden	30			60	
Dauer des max. Drucks in Std.	2	3	4	1	2
Ausbeute in %	78,31	76,93	74,11	74,46	70,46
	In % der Trockensubstanz				
Verd. Alkali-Extrakt	31,27	30,85	30,48	31,87	31,63
Lignin	21,91	21,28	20,83	21,75	21,74
Pentosan	14,46	15,07	12,83	11,67	7,79
Asche	3,38	2,25	1,69	2,35	1,59
Cellulose	48,16	46,71	45,34	44,66	42,28
In % der Cellulose					
α -Cellulose	81,84	82,19	82,85	85,04	88,20
β -Cellulose	16,94	16,41	15,25	13,77	10,26
γ -Cellulose	1,22	1,41	1,90	1,19	1,54

Die Ergebnisse der Aufschlußversuche mit dieser vorbehandelten Bagasse sind in Tabelle XIII zusammengefaßt.

Tabelle XIII.

		Dauer des max. Drucks 2 Stunden						
Max. Druck in Atm.	NaOH in %	Ausbeute in %	Pentosan	Lignin	Asche	Cellulose	α -Cellulose	
			In % des Zellstoffs					
5	{	6	43,3	7,80	2,40	1,89	92,09	90,10
		4	45,5	11,29	3,17	1,77	94,35	92,04
		3	48,7	13,43	3,84	1,69	93,51	91,74
		2	51,5	14,55	5,61	1,89	90,17	89,14
6	{	6	42,1	7,64	1,72	1,12	93,65	86,94
		4	44,3	10,99	2,21	1,09	93,66	88,62
		3	47,2	12,07	2,51	1,34	92,46	88,96
		2	51,6	14,05	4,48	1,08	91,30	87,74
6,5	{	6	40,2	7,70	1,68	1,28	91,87	86,01
		4	41,4	9,91	2,19	1,79	93,63	88,05
		3	44,5	10,34	2,28	1,92	94,83	88,93
		2	47,3	10,42	3,25	1,90	92,04	88,99

Die Bleichungsversuche wurden in folgender Weise durchgeführt: Der Zellstoff wurde mit dem gleichen Gewicht von Wasser gemischt und eine Stunde bei Zimmertemperatur mit Chlorgas behandelt. Hierauf wurde derselbe mit verdünnter Natriumhydroxydlösung gekocht und dann wieder mit Bleichpulver gebleicht. Der gebleichte Zellstoff wurde auch analysiert (Tabelle XIV).

Tabelle XIV.

Aufschlußbedingungen		Ausbeute nach der Bleichung in %	α -Cellulose	β -Cellulose	γ -Cellulose	Pentosan	Asche	
Max. Druck in Atm.,	NaOH in %							
			In % des Zellstoffs					
6,5	{	6	94,7	88,75	5,41	5,84	9,96	0,88
		4	94,9	89,10	6,21	4,69	13,24	0,76
		3	93,2	87,50	7,29	5,21	13,94	1,04
		2	90,8	82,37	9,13	8,50	15,19	1,15
6	{	6	95,2	89,69	5,30	5,01	9,06	1,08
		4	95,4	89,63	5,71	4,66	10,28	1,01
		3	92,6	86,03	6,20	7,77	11,51	1,13
		2	90,8	82,70	8,70	8,60	14,75	1,09
5	{	6	96,8	90,51	4,21	5,28	7,98	0,98
		4	96,8	91,69	4,63	3,68	9,87	0,86
		3	92,5	91,05	4,71	4,24	11,01	0,85
		2	90,7	89,10	5,70	5,20	12,41	1,01

Influence of Monochromatic Light on the Action of Enzymes.

Epecially Influence of Monochromatic Light on the Action of Yeast Enzymes.

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In order to study the effects of spectral monochromatic light on the action of yeast enzymes, saccharase, proteinase, catalase, amylase, and lipase were extracted by autolyzing "Oriental" pressed yeast, the first three of which were refined as follows. In the case of saccharase, after the liquefaction was completed by the addition of 10% of toluene to 225 g of the yeast, the same volume of water was added and the whole allowed to autolyze at room temperature for 7 days. The precipitate obtained by adding an equal volume of alcohol to the autolyzed liquid was extracted with 20% alcohol. Alcohol was then added to the extract, and the saccharase extracted from the second precipitate with 100 cc of water.

In the case of proteinase, after 250 g of the yeast was liquefied by the addition of 10% of ethyl acetate, 500 cc of water was added and the acid that had formed in 2 hours was neutralized continuously by the addition of ammonia water and then a solution that rendered ineffective the tryptic and ereptic actions was separated out and washed with water. The yeast was then suspended in water containing toluene and allowed to autolyze at room temperature for 24 hours. An acetic solution was added to the autolyzate obtained by filtration, and after the pH of the liquid was made 5.0, a *N*/15 acetate buffer of pH 5.0 and aluminium hydroxide were added. The proteinase was finally obtained by elution of the adsorbate with 44 cc of secondary ammonium phosphate.

Catalase was prepared by liquefying yeast. By adding to 150 g of it 33% of toluene the yeast was liquefied within 1 hour at 40°C, after which 200 cc of water was added, and the whole allowed to autolyze overnight in a refrigerator. To the autolyzate was added 44 cc of *N*/10 hydrochloric acid, and the total volume of liquid made up to 2000 cc with water to which aluminium hydroxide was finally added. The catalase was obtained by elution of the adsorbate with 400 cc of *M*/30 phosphate solution (pH=7.6).

Amylase and lipase were prepared by autolyzing for 1 day at room temperature and then overnight in a refrigerator after adding 10% of toluene and 2.5 times the volume of water to 200 g of the yeast.

As substrate for the saccharase, 20% saccharose dissolved in 1% primary sodium phosphate solution was used. Into a test tube containing 4 cc of the substrate, 1 cc of the enzyme solution was added, and the pH of the resulting solution

adjusted to 4.24. The substrate for the amylase was 1% soluble starch solution. Into a test tube containing 10 cc each of the substrate and phosphate buffer solution, 10 cc of the enzyme solution was added, and the pH of the solution adjusted to 6.71. The substrate for the proteinase was 5% gelatine solution. Into a test tube containing 5 cc each of the substrate and citrate buffer, 1 cc of the enzyme solution was added, the resulting pH of the solution being 5.1. The substrate for the lipase, which was castor oil or olive oil, was neutralized with *N*/20 sodium hydroxide. Into a test tube containing 5 cc of the substrate, 5 cc of the enzyme solution was added, and the pH of the resulting solution adjusted to 7.4. The substrate for catalase was *N*/20 hydrogen peroxide buffered with phosphate mixture, the pH of which was 6.7. One cc of the enzyme solution was added to a test tube containing 20 cc of the substrate. The test tubes were placed within tin boxes, and filters placed at the front windows of the boxes. The boxes containing the test tubes were incubated at from 20° to 40°C, according to the particular enzyme, the door opened, and then lighted by a lamp through filters and a 2 cm layer of *N*/10 copper sulphate solution (except in the case of work on infra-red rays), as the copper solution absorbs infra-red rays from a distance of 1 meter.

As the light source, a nitra lamp (Mazda C 100~300 watt and O. K. 500 watt) was used for visible rays. "Vim Ray" blue and red lamps (each 300 watt) used respectively for ultra-violet and infra-red rays.

The filters were made by spreading 7 cc of gelatine solutions, containing various pigments per 1 square dm, over colourless glass or "Acme ultra vit glass" plates which were used respectively for the purpose of visible and infra-red rays or ultra-violet rays, and then drying by means of a fan. The amounts of pigment per 70 cc of gelatine solution are shown in Table I.

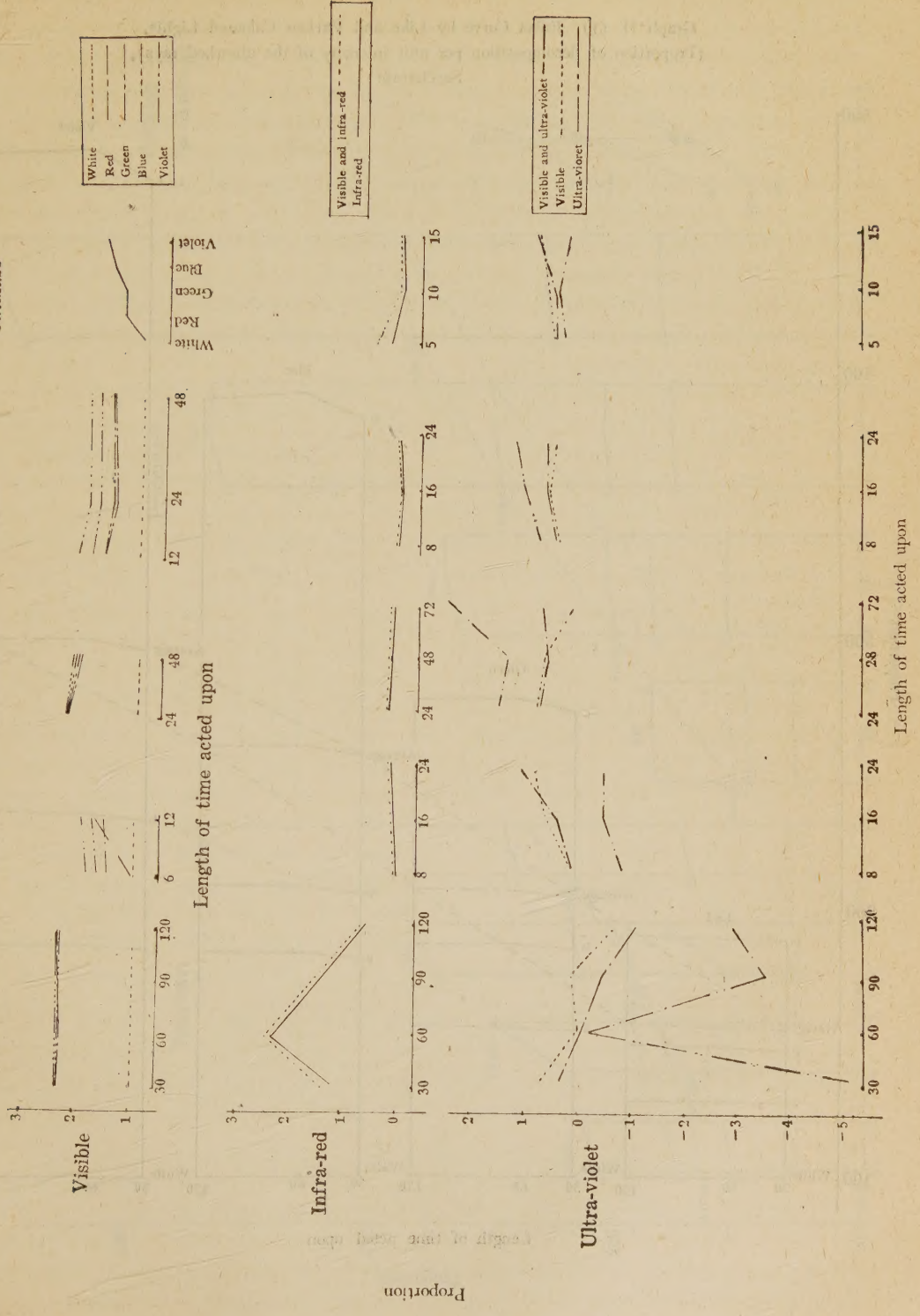
Table I. Composition of the filters.

Plate	Filter	Pigment
Glass plate	White filter	Aesculin 0.2 g or gelatine alone
	Infra-red pass filter	Filter blue 0.1 + filter yellow 0.1 + toluidine blue 0.01 g
	Red filter	Rhodamine 0.42 + tartrazine 0.42 + erythrosine 0.42 g
	Green filter	Patent blue 0.2 + tartrazine 0.7 g
	Blue filter	Patent blue 0.2 + rhodamine 0.7 + aesculin 0.2 g
	Violet filter	Methyl violet 0.42 + toluidine blue 0.17 + aesculin 0.2 g
	Black filter	India ink 3.5 cc
"Acme ultra vit glass" plate	Ultra-violet close filter	Aesculin 0.2 g
	Ultra-violet pass filter	Nitrosodimethylaniline 0.03 + toluidine blue 0.06 + copper sulphate 0.874 g

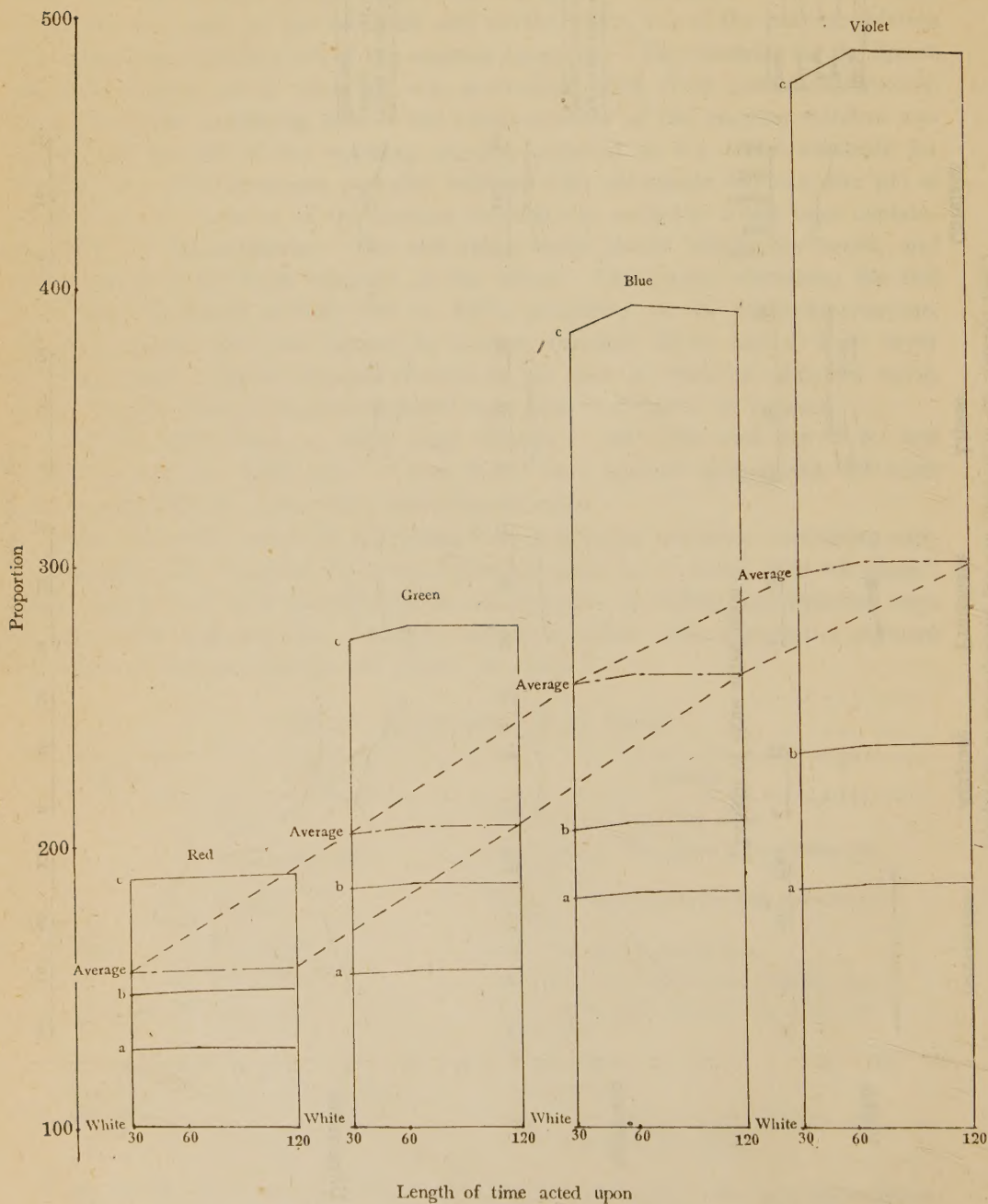
As to the wave lengths of the transmission rays passed through these filters, they were spectroscopically examined; the photograms, which were taken by means of a constant deviation wave length spectrometer and a quartz spectrograph, are

Graph I. Effect Curve by Visible, Infra-red, and Ultra-violet Rays.
(Proportion of decomposition per unit intensity of the absorbed rays.)

Saccharase Amylase Protéïnase Lipase Catalase



Graph II (1) Effect Curve by Like and Various Coloured Lights,
(Proportion of decomposition per unit intensity of the absorbed rays).
Saccharase



shown in Plates I~V. The relative intensities of the transmission rays and the rays absorbed in the substrates or the enzyme solutions containing the substrates were measured by means of a sensitive galvanometer and a vacuum thermojunction, the junction part of which was blackened by carbon, and the determination made in comparison with a Mazda C 100 watt lamp as standard. The intensity per $\text{cm}^2/\text{sec.}$ of a Mazda C 100 watt lamp compared with a Hefner lamp, candle power of which was corrected for temperature, humidity, and atmospheric pressure in the room corresponded to 12.525, and the intensities of the transmission rays of the former passing through the white filter, and the white filter plus a 2 cm layer of $N/10$ copper sulphate, corresponded respectively to 8.017 and 0.422. The total energy per $\text{cm}^2/\text{sec.}$ of the incident light from the Hefner lamp at a distance of 1 meter was 215×10^{-7} gram calory as determined by Ångström. The details are given in the following Table.

Table II. Wave lengths and relative intensities.

Filter	Transmission wave length (I. Å.) Light source -Lamp.	Relative intensity of transmission rays	Relative intensity of the rays absorbed in the substrate or in the enzyme solution containing the substrate				
			Saccharase	Amylase	Proteinase	Lipase	Catalase
Infra-red	White filter	5,700~30,000	2.08	2.18	2.11	2.09	2.08
	Infra-red pass filter	8,000~30,000	2.04	2.13	2.06	2.03	2.05
	Black filter	None	0	0	0	0	0
Visible	White filter	4,000~ 8,000	1.00	1.00	1.00	1.00	1.00
	Red filter	6,200~ 8,000	0.67	0.42	0.63	0.46	0.71
	Green filter	5,170~ 5,400	0.63	0.42	0.60	0.46	0.69
	Blue filter	4,300~ 4,800	0.55	0.42	0.54	0.46	0.61
	Violet filter	4,100~ 4,470	0.48	0.42	0.48	0.46	0.55
	Black filter	None	0	0	0	0	0
Ultra-violet	White filter	3,500~ 5,500	0.33	0.43	0.32	0.40	0.35
	Ultra-violet close filter	4,100~ 5,500	0.32	0.40	0.32	0.37	0.35
	Ultra-violet pass filter	3,500~ 4,000	0.15	0.17	0.18	0.17	0.20
	Black filter	None	0	0	0	0	0

The test tubes were removed from each box at fixed intervals after the incubation and the end products of the action of the enzymes were determined by Bertrand's method for saccharase and amylase, by the formol method for proteinase, by Knitz's method for lipase, and by the permanganate titration method for catalase.

The controls were deducted from the quantitative values. The differences correspond to the real amounts of the substrates decomposed by the action of the enzymes. In the case of visible rays, the index-numbers obtained from the real amounts under each of the various lights other than white, compared with white light as a standard, were divided by the relative intensities of the absorbed rays.

In the case of infra-red and ultra-violet rays, the differences between the black and other rays were divided by the relative intensities of the absorbed rays. Thus, the extent of decomposition caused by the enzymes per unit intensity of the absorbed rays were obtained in both cases. The extent of action of the enzymes under the influence of different lights was compared with the proportions of the quotients that were obtained as already mentioned (See Table V).

The results with the controls deducted from the quantitative values are shown in Table III.

The percentages of the substrates decomposed by the enzymes are given in Table IV.

The extent of decomposition of the substrates by the enzymes, that is to say, the proportions of the quotients per unit intensity of the absorbed rays, are given in Table V.

In this experiment, the actions of saccharase, amylase, proteinase, lipase, and catalase were affected only very slightly by the infra-red rays. This applies to the first-named; the others were scarcely affected by the infra-red rays. The enzymes were more active under rays containing both visible and infra-red. Promotion of enzyme action by visible rays was found to be more pronounced under rays of shorter rather than longer wave length, gradually weakening with increase in wave length. The actions of saccharase, amylase, and catalase were found to be inhibited, contrary to the actions of proteinase and lipase, which were promoted by the near ultra-violet rays. The action of these enzymes irradiated previously by the near ultra-violet or infra-red rays was closely in agreement with the above result. It is therefore believed that the limits to the actions of saccharase, amylase, and catalase lie near the ultra-violet region, close to the violet, that is to say, the actions of these enzymes were promoted or inhibited under wave lengths either longer or shorter than that from a certain point in the near ultra-violet region. In the cases of proteinase and lipase, seeing that the activities of the two enzymes were found to increase more with near ultra-violet rays than with the visible rays, it cannot be said that their activities had reached their limits.

The writer made further investigations into the effect of visible rays on the action of these enzymes, using series of like and different coloured lights consisting of red, green, blue, and violet, each in three or four different wave lengths and intensities. The light source and the means of eliminating the infrared and ultraviolet rays were the same as already described. The transmission wave lengths through the filters and the relative intensities of the absorbed rays in the substrates or the enzyme solutions containing the substrates are given in Table VI. See also plates VI, VII.

The other methods used, except those of the filters held at the front of the boxes were also the same as those already described.

The results, with the controls deducted from the quantitative values are shown in Table VII.

The percentages of the substrates decomposed by the enzymes are given in a Table VIII.

Table III. The net quantitative values per test tube.

Enzyme	Saccharase	Amylase		Proteinase		Lipase		Catalase				
		(30)	(60)	minute (120)	hour (8)	hour (16)	hour (24)	hour (24)	hour (16)	hour (8)	hour (24)	minute (15)
Ray	Visible and infra-red											
	Infra-red	607.50	744.75	829.50	920.00	1.65	2.64	2.33	1.73	2.31	2.67	25.09
	Black	601.38	741.75	823.25	917.75	1.60	2.56	2.24	1.68	2.26	2.61	25.09
		563.63	662.38	777.25	895.38	1.58	2.37	1.98	1.58	2.20	2.60	24.55
White						(6)	(12)					(48)
	Red	467.63	639.88	743.00	820.38	8.72	37.54		1.02	1.37	15.94	24.69
	Green	463.88	634.38	733.75	821.13	8.32	37.21		1.05	1.30	17.07	23.70
	Blue	465.38	635.50	731.88	816.75	8.26	36.79		1.05	1.32	15.94	23.00
	Violet	465.13	635.13	733.00	817.63	8.13	36.24		1.06	1.33	16.33	23.85
	Black	465.25	637.75	742.63	824.13	7.80	36.01		1.06	1.35	15.94	22.86
		451.00	626.50	724.00	812.38	7.13	34.69		0.96	1.16	14.11	21.38
						(8)	(16)			(8)	(16)	(24)
	Visible and ultra-violet											
	Visible	334.00	491.25	589.75	647.13	1.44	3.41	3.24	2.32	2.92	3.12	24.98
Ultra-violet	336.25	491.85	593.50	651.88	1.46	3.48	3.14	2.32	2.92	3.04	25.53	
Black	317.88	491.00	583.13	647.00	1.23	3.16	2.98	2.33	2.94	3.20	25.88	
	331.50	491.55	592.63	656.74	1.42	3.28	2.84	2.24	2.87	3.07	23.94	

Table IV. Percentage of substrates decomposed by the enzymes.

Enzyme	Saccharase			Amylase		Proteinase		Lipase		Catalase				
Length of time acted upon	(30)	(60)	(90)	minute (120)	hour (8)	hour (16)	hour (24)	hour (48)	hour (72)	hour (8)	hour (16)	hour (24)	hour (24)	minute (24)
Ray														
Visible and Infra-red	36.14	44.82	50.27	56.08	1.34	2.14	1.89	4.32	5.79	6.68	0.49	0.73	0.81	27.38
Infra-red	35.78	44.62	49.89	56.00	1.30	2.08	1.82	4.20	5.65	6.52	0.48	0.72	0.81	26.90
Black	33.39	39.65	46.92	54.54	1.27	1.93	1.61	3.94	5.50	6.52	0.47	0.72	0.79	26.32
					(6)	(12)					(12)	(24)	(48)	37.21
White	27.50	38.24	44.70	49.69	6.97	30.55		2.55	3.42		0.52	0.80	1.22	51.05

Red	27.28	37.90	44.12	49.74	6.64	30.28	2.61	3.26	0.55	0.77	1.21	51.00
Green	27.37	37.97	44.00	49.47	6.60	29.93	2.62	3.29	0.52	0.74	1.19	50.74
Blue	27.35	37.95	44.08	49.52	6.49	29.48	2.64	3.33	0.53	0.78	1.18	50.54
Violet	27.36	38.11	44.68	49.86	6.22	29.28	2.66	3.37	0.52	0.74	1.15	50.19
Black	26.38	37.40	43.57	49.19	5.67	28.18	2.41	2.91	0.46	0.69	1.12	48.70
					(8)	(16)			(8)	(16)	(24)	
Visible and ultra-violet	19.53	28.91	35.03	38.82	1.16	2.77	5.80	7.32	0.54	0.74	0.81	31.59
Visible	19.63	28.94	35.26	38.99	1.18	2.82	5.80	7.31	0.54	0.73	0.77	31.59
Ultra-violet	18.48	28.89	34.61	38.81	0.99	2.56	5.84	7.36	0.55	0.74	0.83	31.38
Black	19.34	28.95	35.21	38.30	1.15	2.66	5.61	7.18	0.51	0.67	0.75	31.56
												39.86
												48.94

Table V. Proportion of decomposition per unit intensity of the absorbed rays.

Enzyme	Saccharase			Amylase		Proteinase		Lipase			Catalase				
	(30)	(60)	(90)	minute (120)	(8)	(16)	hour (24)	(48)	hour (72)	(8)	(16)	hour (24)	(15)	(10)	minute (15)
Ray															
Visible and infra red	1.26	2.37	1.54	0.71	0.03	0.10	0.14	0.18	0.14	0.008	0.004	0.009	0.51	0.10	0.07
Infra-red	1.11	2.34	1.40	0.68	0.01	0.07	0.10	0.13	0.07	0.005	0.003	0.009	0.28	0.03	0.02
Black	—	—	—	—	(6)	(12)	—	—	—	(12)	(24)	(48)	—	—	—
White	1.00	1.00	1.00	1.00	1.00	1.00	—	1.00	1.00	1.00	1.00	1.00	1.00	—	—
Red	2.36	2.38	2.35	2.38	1.50	1.57	—	2.23	2.08	1.61	1.44	1.49	1.41	—	—
Green	2.37	2.36	2.35	2.37	1.07	1.63	—	2.23	2.11	1.61	1.49	1.56	1.44	—	—
Blue	2.37	2.36	2.35	2.37	1.71	1.77	—	2.25	2.14	1.87	1.77	1.76	1.64	—	—
Violet	2.37	2.37	2.38	2.39	1.86	2.00	—	2.27	2.17	2.09	1.93	1.98	1.78	—	—
Black	—	—	—	—	(8)	(16)	—	—	—	(8)	(16)	(24)	—	—	—
Visible and ultra-violet	0.37	(-)	0.05	(-)	0.05	0.35	1.00	0.48	0.34	0.12	0.26	0.23	0.03	0.03	0.27
Visible	0.73	0.03	0.13	(-)	0.09	0.51	0.74	0.51	0.35	(-)	0.20	0.10	0.03	0.10	0.24
Ultra-violet	(-)	0.24	(-)	0.24	(-)	0.87	(-)	1.37	1.08	0.43	0.69	0.82	(-)	0.02	(-)
Black	—	—	—	—	(-)	0.54	0.59	—	—	—	—	—	—	—	—

Table VI. The wave lengths and the relative intensities.

Filter	Transmission wave length (I. Å.) Light source—Lamp.	Relative intensities of the absorbed rays			
		Saccharase	Amylase	Proteinase	Catalase
White	4,000~8,000	1.00	1.00	1.00	1.00
Red a	6,000~8,000	0.78	0.78	0.79	—
b	6,050~8,000	0.67	0.63	0.68	0.71
c	6,130~8,000	0.52	0.52	0.54	0.59
d	6,230~8,000	—	0.42	—	0.49
Green a	5,000~5,700	0.63	0.76	0.64	—
b	5,140~5,500	0.53	0.60	0.53	0.69
c	5,170~5,400	0.35	0.48	0.37	0.56
d	5,200~5,400	—	0.38	—	0.46
Blue a	4,200~4,960	0.54	0.74	0.56	—
b	4,270~4,900	0.47	0.54	0.49	0.61
c	4,300~4,800	0.25	0.40	0.27	0.46
d	4,380~4,700	—	0.30	—	0.35
Violet a	4,000~4,530	0.53	0.69	0.54	—
b	4,100~4,470	0.42	0.48	0.43	0.55
c	4,150~4,400	0.20	0.33	0.22	0.40
d	4,150~4,300	—	0.23	—	0.28
Black	None	0	0	0	0

Table VII. The net quantitative values per test tube.

Remark: At amylase, one series marked Δ and other series unmarked.

Enzyme		Saccharase			Amylase		Proteinase			Catalase
Ray	Length of time acted upon	mg of reduced copper			mg of reduced copper		mg of nitrogen decomposed			mg of H_2O_2 decomposed
		minute			hour		hour			minute
		30	60	120	6	12	24	48	72	15
White		552.50	746.38	871.88	35.88 Δ 12.47	44.48 Δ 56.78	3.60	4.93	6.26	8.89
Red	a	547.50	742.88	874.75	35.25 Δ	44.18 Δ	3.59	4.92	6.24	—
	b	543.75	739.50	870.00	12.16	56.39	3.56	4.90	6.21	8.89
	c	540.25	725.38	864.13	32.82 Δ	41.47 Δ	3.53	4.88	6.21	8.79
	d	—	—	—	10.75	54.09	—	—	—	8.69
Green	a	543.13	738.75	868.63	34.66 Δ	41.70 Δ	3.56	4.89	6.21	—
	b	540.50	736.00	864.25	11.22	54.98	3.53	4.88	6.21	8.79
	c	533.50	732.25	856.88	32.11 Δ	40.69 Δ	3.50	4.85	6.14	8.70
	d	—	—	—	10.19	53.48	—	—	—	8.61
Blue	a	540.88	736.75	864.50	33.59 Δ	40.76 Δ	3.54	4.89	6.21	—
	b	538.75	734.88	862.25	10.37	54.50	3.53	4.87	6.19	8.75
	c	529.63	731.63	852.13	30.32 Δ	39.09 Δ	3.46	4.83	6.12	8.65
	d	—	—	—	9.90	52.96	—	—	—	8.50
Violet	a	540.75	736.25	864.38	32.80 Δ	39.49 Δ	3.53	4.88	6.20	—

b	536.75	733.00	859.88	10.03	52.75	3.51	4.86	6.17	8.64
c	528.00	729.75	850.13	28.26 Δ	36.19 Δ	3.45	4.82	6.09	8.45
d	—	—	—	9.20	49.36	—	—	—	8.35
Black	516.13	717.38	840.13	27.71 Δ	35.36 Δ	3.37	4.75	5.94	8.11
				9.02	48.23				

Table VIII. The percentages of substrates decomposed by the enzyme.

Enzyme		Saccharase			Amylase		Proteinase			Catalase
Length of time acted upon		minute			hour		hour			minute
Ray		30	60	120	6	12	24	48	72	15
White		32.69	44.93	53.07	29.15 Δ	36.32 Δ	9.01	12.33	15.65	52.30
					10.04	46.59				
Red	a	32.38	44.69	53.24	28.39 Δ	36.06 Δ	8.97	12.32	15.60	—
	b	32.15	44.48	52.92	9.79	46.26	8.91	12.26	15.55	52.24
	c	31.93	44.22	52.54	26.61 Δ	33.81 Δ	8.84	12.21	15.53	51.66
	d	—	—	—	8.63	44.34	—	—	—	51.09
Green	a	32.12	44.42	52.83	28.15 Δ	34.00 Δ	8.90	12.25	15.54	—
	b	31.94	44.26	52.55	9.02	45.07	8.84	12.21	15.53	51.70
	c	31.51	44.02	52.06	26.03 Δ	33.16 Δ	8.75	12.13	15.38	51.18
	d	—	—	—	8.18	43.84	—	—	—	50.65
Blue	a	31.97	44.30	52.57	27.25 Δ	33.68 Δ	8.85	12.24	15.54	—
	b	31.83	44.18	52.42	8.32	44.68	8.83	12.19	15.48	51.48
	c	31.27	43.98	51.75	24.57 Δ	31.84 Δ	8.67	12.08	15.31	50.89
	d	—	—	—	7.93	43.41	—	—	—	49.95
Violet	a	31.97	44.27	52.55	26.59 Δ	32.15 Δ	8.84	12.22	15.53	—
	b	31.72	44.07	52.26	8.04	43.24	8.79	12.16	15.43	50.82
	c	31.17	43.87	51.62	22.92 Δ	29.42 Δ	8.63	12.05	15.25	49.67
	d	—	—	—	7.36	40.47	—	—	—	48.97
Black		30.42	42.97	50.95	22.47 Δ	28.70 Δ	8.42	11.88	14.87	47.66
					7.22	39.54				

The index-numbers for decomposition under each coloured light compared with that under white light as standard is shown in the following Table.

Table IX. The index-numbers for decomposition under each light.

Enzyme		Saccharase			Amylase		Proteinase			Catalase
Length of time acted upon		minute			hour		hour			minute
Ray		30	60	120	6	12	24	48	72	15
White		100.00	100.00	100.00	100.00 Δ	100.00 Δ	100.00	100.00	100.00	100.00
					100.00	100.00				
Red	a	99.10	99.53	100.33	98.18 Δ	99.31 Δ	99.53	99.77	99.69	—
	b	98.42	99.08	99.79	97.57	99.31	98.87	99.32	99.33	99.90

Green	c	97.78	98.53	99.11	91.75 Δ	93.27 Δ	98.06	98.95	99.22	98.88
	d	—	—	—	87.20	90.33	—	—	—	97.75
	a	98.30	98.98	99.63	96.68 Δ	93.83 Δ	98.72	99.21	99.31	—
	b	97.83	98.61	99.13	89.27	97.55	98.06	98.95	99.22	98.88
Blue	c	96.56	98.11	98.28	89.62 Δ	91.54 Δ	97.04	98.24	98.23	97.86
	d	—	—	—	82.45	94.32	—	—	—	96.85
	a	97.90	98.71	99.15	93.54 Δ	92.73 Δ	98.18	99.03	99.26	—
	b	97.51	98.46	98.90	84.12	96.87	97.94	98.75	98.93	98.43
Violet	c	95.86	98.02	97.74	85.06 Δ	88.00 Δ	96.15	97.84	97.81	97.30
	d	—	—	—	80.56	92.88	—	—	—	95.61
	a	97.87	98.64	99.14	91.32 Δ	89.36 Δ	98.13	98.98	99.19	—
	b	97.15	98.21	98.62	81.34	93.30	97.55	98.52	98.59	97.19
Black	c	95.57	97.77	97.51	79.13 Δ	81.41 Δ	95.72	97.64	97.42	95.05
	d	—	—	—	76.08	85.96	—	—	—	93.93
		93.41	96.12	96.36	77.58 Δ	79.54 Δ	93.43	96.25	95.03	91.23
					74.59	83.98				

This result confirms that the actions of the yeast saccharase, amylase, proteinase, and catalase were promoted by visible monochromatic lights, which was found to be the stronger the greater the relative intensities of the absorbed rays under the same coloured lights.

The numerical values, namely the index-numbers divided by the relative intensities of the absorbed rays, that is, the proportions of the enzymic decomposition per unit intensity of each light are shown in the following Table.

Table X. Proportion of decomposition per unit intensity.

Enzyme		Saccharase			Amylase		Proteinase			Catalase
Ray	Length of time acted upon	minute			hour		hour			minute
		30	60	120	6	12	24	48	72	15
	White	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Red	a	127.4	127.9	129.0	126.4	127.8	126.8	127.1	127.1	—
	b	147.6	148.4	149.6	153.9	156.6	145.8	146.5	146.5	139.3
	c	188.4	189.8	191.0	176.7	180.3	183.3	185.0	185.0	168.0
	d	—	—	—	206.6	214.1	—	—	—	199.9
	Average	154.4	155.4	156.5	165.9	169.7	152.0	152.9	152.9	169.1
Green	a	155.1	156.1	156.4	127.0	123.3	154.0	154.8	154.8	—
	b	185.3	186.8	187.7	148.4	162.4	184.0	185.7	185.7	143.8
	c	274.3	278.7	279.2	188.7	192.7	261.2	267.7	267.7	173.2
	d	—	—	—	219.3	250.8	—	—	—	207.4
	Average	204.9	207.2	207.8	170.9	182.3	199.7	202.7	203.2	174.8
Blue	a	182.0	183.5	184.3	126.4	125.3	175.6	177.3	177.6	—
	b	206.6	208.6	209.5	154.9	178.4	201.9	203.6	204.0	163.3
	c	383.4	392.1	390.9	212.1	219.4	356.1	362.4	362.2	209.4

	d	—	—	—	272.1	281.7	—	—	—	264.9
Average		257.3	261.4	261.4	191.4	201.2	244.6	247.8	247.9	212.5
Violet	a	185.4	186.8	187.8	132.3	129.1	180.4	181.9	182.3	—
	b	233.0	235.5	236.5	169.8	194.8	225.3	227.6	227.7	202.8
	c	470.8	481.6	480.3	238.3	245.2	437.1	445.9	444.9	235.1
	d	—	—	—	334.2	375.4	—	—	—	324.3
Average		296.4	301.3	301.5	218.7	236.1	280.9	285.1	285.0	254.1
Black		—	—	—	—	—	—	—	—	—

According to these experiments, the extent of enzyme action per unit intensity by the same coloured light of saccharase, amylase, proteinase, and catalase was found to increase with reduction in the relative intensities of the absorbed rays, but the effect per unit intensity of the various coloured lights on the action of these enzymes was found to be approximately proportional to the wave number of the absorbed rays.

As to the cause of the inhibition of enzyme action due to the lights, GORBACH and LEACH (1930) from their studies on saccharase, attributed it to some hitch in the working of the tryptophane, the carrier of an active group in the enzyme. The former investigator and PICK (1932) later reported that inactivation of the enzyme with the aid of ultra-violet rays was greater in the presence of ozone, in contrast to its usual activation in the presence of molecular oxygen. LINDNER (1922; 1927) pointed out that this was more advantageous for fermentation owing to the vitamins formed in the yeast bodies through the action of the ultra-violet rays. According to GREEN (1897), in his work on amylase, the cause of increase in saccharification with the aid of rays, such as infra-red, red, orange, and blue, was the changes in some of the zymogen to an active enzyme.

The opinion of the writer is that the stable state E of the enzyme molecules changes to the active state E' through radiant energy ($h\nu$) possessing suitable vibration, when the action on the substrate S becomes pronounced and changes S to the labile state S' , that is

$$E + h\nu = E'$$

$$E' + S = S'.$$

He also believes that when the end product increases, it means that the action is promoted through the lights, it being clear that the promotion was found to be stronger, the greater the relative intensities of absorbed rays under the same coloured visible lights. If, however, the enzyme happens to meet with radiant energy possessing unsuitable vibration, it becomes to the destructive state E'' , owing to photolysis, that is

$$E + h\nu = E''$$

and when the decomposition of the substrate is diminished it has an inhibiting effect on the action.

Needless to say, if enzyme enter into special substrate, of which a portion of the molecules kept the state of the stable E naturally, it can be changed to the active state E' at a certain temperature without radiant energy, and then changes a portion of S by the action of E' to the labile state S' . The change of E to E' increases as a temperature rise to optimum; consequently it increases a possibility to change S to S' .

In case the enzyme received radiant energy possessing suitable vibration, E' is increased, and it changes S plentifully to S' . Thus, decomposition of substrate advances more by the action of the enzyme receiving suitable radiant energy than by the opposite and it represents promotive effect.

If, however, the enzyme happens to meet with the radiant energy possessing a too great and unsuitable vibration, E changes to E'' , and the enzyme is destroyed. Thus, the action of enzyme on substrate becomes weaker than that which has not received radiant energy and it represents an inhibiting effect.

It is shown in such relation as

$$E''_{(min)} > E' > E$$

$E''_{(min)}$ is the state reached the acting limit, as seen in the case of radiant energy in the near ultra-violet region on saccharase, amylase and catalase. The region of wave length to reach E'' varies with the kinds of enzymes. It is seen that E' is plentifully formed, owing to the radiant energy in the near ultra-violet on proteinase and lipase, in spite of E not changing to E'' except in the region of shorter wave length.

The increase of the promotive effect under the same coloured visible lights with increase in the relative intensities of the absorbed rays, depends upon E' being abundantly formed along with the relative intensities, and also the effect per unit intensity of various coloured visible lights is due to E' being formed approximately proportional to wave number of the absorbed rays.

In conclusion, the writer wishes to express his thanks to Prof. U. SUZUKI for his interest and encouragement, and also to the Imperial Academy for grants given in aid of this study.

ABSTRACTS

from

TRANSACTIONS published in JAPANESE

(Pages refer to the Japanese originals of this volume unless otherwise noticed)

Über Vitamin C im Tee.

(SS. 265~270)

Von Akiji FUJITA und Isamu NUMATA.

(Biochemisches Laboratorium des Kitasato-Institutes, Tokyo; Eingegangen am 19. 3. 1940.)

On the Carbohydrate in Hen's Ovovitellin.

(pp. 271~276)

By Kinsuke KONDO and Uichiro SARATA.

(Nutritional Chemical Laboratory, Faculty of Agriculture and Chemical Institute, Kotyo Imperial University; Received Mar. 6, 1940.)

Die Abhängigkeit der Enzymentwicklung, insbesondere von Amylase und Protease, von der Art der Gerste und ihrer Keimung.

(SS. 277~280)

Von H. NAKAMURA.

(Aus dem Laboratorium der Dainippon Brauerei; Eingegangen am 27. Feb. 1940.)

1) Die Entwicklung von Amylase und Protease bei der Keimung ist abhängig von der Art der Gerste, wodurch auch das Verhältnis der bezüglichen Enzymkräfte untereinander sehr verschieden sein kann.

<i>Beispiel:</i>	<i>Wurzellänge bezogen auf Kornlänge:</i>	<i>Protease:</i>	<i>Amylase:</i>
<i>Gerste A:</i>	1,6	5,3	3,2
<i>Gerste B:</i>	1,6	2,3	7,7

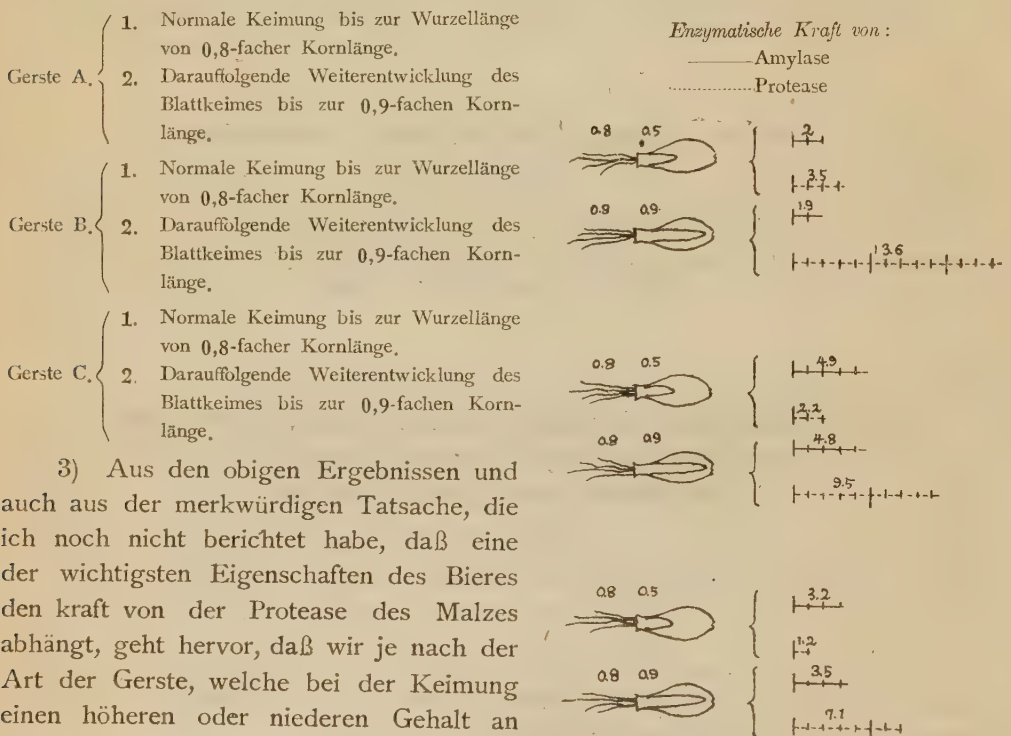
Wenn bei der Gerste B die Amylasenwirkung zur Proteasewirkung mit 1:1 gesetzt wird, so ergibt sich bei der Gerste A die Relation der gleichen Wirkung mit 1:5,5.

2) Wenn der Wurzelkeim nach Erreichung einer bestimmten Länge im Wach-

sen behindert wird, der Blattkeim sich aber weiterentwickelt, dann hört die Bildung der Amylase ganz auf, dagegen nimmt die Protease in einem erstaunlichen Umfang zu.

Falls beispielsweise der Wurzelkeim einer Gerstenart bei der Keimung nur bis zur 0,8-fachen Kornlänge wächst und der Blattkeim daraufhin bis zur 0,9-fachen Kornlänge vorgetrieben wird, so tritt in der Bildung von Amylase ein Stillstand ein, wogegen die Protease eine weitere Zunahme—und zwar um das 4-bis 6-fache—erfährt. Dementsprechend ist dann auch die enzymatische Kraft des nach diesem Verfahren erreichten Proteasegehaltes eine vielfach größere als die beim Höchstgehalt an Protease, welche bei der natürlich gekeimten Gerste entsteht.

Bei Anwendung dieses Verfahrens auf verschiedene Gerstenarten unterliegen die absoluten und relativen Werte von Amylase und Protease weitgehenden Schwankungen.



3) Aus den obigen Ergebnissen und auch aus der merkwürdigen Tatsache, die ich noch nicht berichtet habe, daß eine der wichtigsten Eigenschaften des Bieres den kraft von der Protease des Malzes abhängt, geht hervor, daß wir je nach der Art der Gerste, welche bei der Keimung einen höheren oder niederen Gehalt an Protease entwickelt, bestrebt sein müssen, diese Bildung an Protease genau zu überwachen und zu lenken. Die Erreichung dieses Zieles setzt die Ausarbeitung entsprechender neuer Kontrollverfahren voraus.

On Alcoholic Fermentation of Acorn by Amylo Process.

(pp. 281~287)

By Seisaku SUGIZAKI.

(Agricultural Chemical Laboratory, Department of Agriculture, Tokyo Imperial University;

Received March 6, 1940.)

Studies on the Vegetable Tannins in Taiwan. Part 5.

Manufacture of Tanning Extract from the Bark of *Acacia confusa*. I.

(pp. 288~292)

By Yasuyosi OSIMA, Minoru ISII and Zenyu HYO.

(Agricultural Chemical Department, Taihoku Imperial University, Taiwan;

Received March, 19, 1940.)

We found that the maximum yield of tannin was obtained by extracting the dried bark with 50% alcohol. From fresh bark extraction with water gives equally good yield but the extraction was far easier and very much faster with 50% alcohol.

The maximum temperatures for extraction of the materials are as follows:

Dried bark extracted with water	80°C.
" " 50% alcohol	60~80°C.
Fresh bark " "	80°C.

On a New Polypeptide Isolated from *Eisenia Bicyclis*.

(Part III)

A Study of the Chemical Structure of Eisenin. (2)

(pp. 293~298)

By Tosihiko OOHIRA.

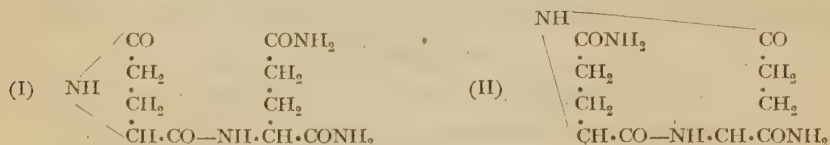
(Agricultural Chemical Laboratory, Tokyo Imperial University;

Received Mar. 12, 1940.)

For the determination of the amino acid having a free carboxyl group in eisenin molecule, eisenin was converted into thiohydantoin derivative by means of ammonium thiocyanate and acetic acid anhydride; then it was decomposed by 25% aqueous ammonia solution, according to the method by P. Schlack and W. Kumpf. The ammonia was removed from the solution by evaporation under reduced pressure, and from the residue two different crystals A [m. p. 160~161°] and B [m. p. 240~241° (decomp.)] were obtained; the "A" from the ether extract and the "B" from the residue. The "A" was identified as 5-methyl-2-thiohydration by com-

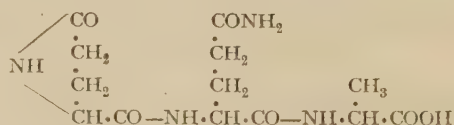
paring with an authentic specimen prepared from alanine. The "B" was found to be a new dipeptide $C_{10}H_{16}O_4N_4$ having two acid-amide groups.

The formation of 5-methyl-2-thiohydantoin from eisenin shows that alanine is in the end position of eisenin molecule; its amino group only being substituted for the peptide grouping. Therefore it is suggested that the chemical structure of the substance, obtained by partial hydrolysis with 3% aqueous bariumhydroxide solution as has been mentioned in the previous paper, may be di- $[\alpha$ -amino- γ -carboxy-butyryl]-alanine. It is presumed also that the dipeptide $C_{10}H_{16}O_4N_4$, mentioned above, may correspond to one of the two structures shown below.



To settle this question, the author prepared *l*-pyroglutamyl-*d*-glutamic acid diamide representing the structure (I), *l*-pyroglutamic acid chloride (1 mol) obtained by the action of thionylchloride on *l*-pyroglutamic acid, was treated with *d*-glutamic acid diethylester (2 mols) in chloroform solution. The reaction mixture was successively washed with dilute hydrochloric acid and aqueous sodium bicarbonate solution and then the solvent was distilled off. The residual crystalline mass was dissolved in a little alcohol and mixture of ether and petroleum ether added to it. The crude crystals of *l*-pyroglutamyl-*d*-glutamic acid diethylester separated out, were recrystallized from acetic ester. This diethylester was treated with 25% aqueous ammonia solution at room temperature for about two hours. After the solution was evaporated to a small volume at about 30~40° under diminished pressure, absolute alcohol was added to the residue until a crystal was separated out by stirring. It was recrystallized from dilute alcohol. This crystal was proved to be completely identical with the substance obtained from thiohydantoin derivative of eisenin by the treatment with ammonia.

From these results, *l*-pyroglutamyl-*d*-glutamyl-*d*-alanine may be given as the chemical structure of eisenin.



Sterilizing Action of Phenols.

Synopsis.

(pp.299 ~305)

By Sogo TETSUMOTO.

(Government Institute for Infect. Dis., Tokyo Imper. University; Received Feb. 27, 1940.)

I reported previously concerning the sterilizing action of mineral acids and fatty acids. Then I studied the sterilizing action of phenol group as noted in the following table.

I. REAGENTS.

TABLE 1. Phenols.

Number of OH	Phenols	Rational formulae	M. W.	weight % at N/1000
1	Phenol	C_6H_5OH	94.048	0.0094
	Cresol (<i>o</i>)	$C_6H_4 \begin{cases} OH \dots \dots (1) \\ CH_3 \dots \dots (2) \end{cases}$	108.064	0.0108
	Guaiacol	$C_6H_3 \begin{cases} OH \dots \dots (1) \\ OCH_3 \dots \dots (2) \end{cases}$	124.064	0.0124
	Thymol	$C_6H_3 \begin{cases} OH \dots \dots (1) \\ -CH(CH_3)_2 \dots (2) \\ CH_3 \dots \dots (3) \end{cases}$	162.167	0.0162
	Picric acid	$C_6H_2(NO_2)_3 \cdot OH$	215.044	0.0215
2	Pyrocatechin (<i>o</i>)	$C_6H_2 \begin{cases} OH \dots \dots (1) \\ OH \dots \dots (2) \end{cases}$	110.048	104°
	Resorcine (<i>m</i>)	$C_6H_4 \begin{cases} OH \dots \dots (1) \\ OH \dots \dots (3) \end{cases}$	"	110°
	Hydroquinone (<i>p</i>)	$C_6H_4 \begin{cases} OH \dots \dots (1) \\ OH \dots \dots (4) \end{cases}$	"	170°
3	Pyrogallie acid (<i>o</i>)	$C_6H_3 \begin{cases} OH \dots \dots (1) \\ OH \dots \dots (2) \\ OH \dots \dots (3) \end{cases}$	126.048	132°
	Phloroglucine (<i>m</i>)	$C_6H_3 \begin{cases} OH \dots \dots (1) \\ OH \dots \dots (2) \\ OH \dots \dots (4) \end{cases}$	"	218°
	Hydroxyhydroquinone (<i>p</i>)	Hydroxyhydroquinon, was not available and the experiment was not performed.		

II. STERILIZING ACTION OF PHENOLS AT THE SAME CONCENTRATION.

The sterilizing power and special character of phenols at the same concentration, were studied in this experiment, and the results obtained are shown in the following table.

Picric acid, cresol and thymol are insoluble at N/100, so they were used in this experiment at N/1000. Only phenol was tested at N/10 and N/100.

TABLE 2. Sterilizing Action of Phenols at the Same Concentration.
N/1000. (Phenol. N/10, N/100) 20°C.

Number of OH	Phenols	Conc.	pH	Surviving period			
				<i>Staph. pyogen.</i>	<i>Prot. vulgar.</i>	<i>Bac. typhos.</i>	<i>Vib. choler.</i>
1	Phenol	N/10	5.17	5 ^m +10 ^m —	1 ^m +2.5 ^m —	2.5 ^m ± 5 ^m —	1 ^m —
	"	N/100	5.21	4 ^d + 5 ^d —	2 ^d + 3 ^d —	4 ^d ± 5 ^d —	90 ^m + 2 ^h —
	"	N/1000	5.45	7 ^d ± 8 ^d —	4 ^d + 5 ^d —	5 + 6 —	9 ^h ± 12 ^h —
	Cresol (o)	"	5.36	6 + 7 —	4 ± 5 —	5 + 6 —	6 ± 9 —
	Guaiacol	"	6.13	10 + 12 —	7 + 8 —	8 + 10 —	24 ± 36 —
	Thymol	"	"	2 ^h + 3 ^h —	60 ^m ±90 ^m —	90 ^m + 2 ^h —	1 ^m ±2.5 ^m —
	Picric acid	"	2.30	90 ^m + 2 ^h —	30 + 45 —	60 ± 90 ^m —	1 ± 2.5 ^m —
2	Pyrocatechin (o)	"	5.31	18 ^h + 24 ^h —	9 ^h ± 12 ^h —	12 ^h + 18 ^h —	10 ^m ±15 ^m —
	Resorcine (m)	"	5.57	13 ^d + 15 ^d —	8 ^d + 12 ^h —	10 ^d + 12 ^d —	24 ^h ± 15 ^m —
	Hydroquinone (p)	"	5.64	12 ^h + 18 ^h —	6 ^h + 9 ^d —	9 ^h + 12 ^h —	15 ^m ± 20 ^m —
3	Pyrogalllic acid (o)	"	4.58	2 ^d + 3 ^d —	18 ^h + 28 ^h —	1 ^d + 2 ^d —	30 ± 45 ^m —
	Phloroglucine (m)	"	5.71	20 ~ 25	10 ^d ~ 15 ^d	15 ~ 20	24 ^h + 36 ^h —
Control				8 ^d ±	5 ^d ±	6 ^d ±	18 ^h ±

From the results noted in table 2 we know the following facts:—

The sterilizing action of phenol, which is usually used as the most popular disinfectant in hygiene, is strong only at N/10, but at lower concentrations the sterilizing action diminishes very distinctly, and at N/1000 concentration phenol has no sterilizing power on bacteria except for *Vib. cholerae*.

The order of the strength of the sterilizing action at the same concentration of phenols is as follows:

Picric acid > Thymol > Hydroquinone > Pyrocatechin > Pyrogalllic acid. Cresol, guaiacol, resorcine and phloroglucine have totally no bactericidal action at N/1000. Especially resorcine and phloroglucine have a remarkable promoting action on the life of the microorganisms.

Relation between the chemical constitution and the strength of the sterilizing action is as follows. From the results of 2(OH)phenols and 3(OH) phenols, we see the following order as to the sterilizing power.

Para > Ortho > Meta.

III. STERILIZING ACTION OF PHENOL SALTS AND PHENOL ANIONS.

The action of phenol salts and of phenol anions on the life of bacteria were tested in this experiment. Concentration of Na, Ca and NH₄ salts, having the same anions as phenols was made N/1000.

TABLE 3. I. Na-salts.

Na-	Surviving period			
	<i>Staph. pyogen.</i>	<i>Prot. vulgar.</i>	<i>Bac. typhos.</i>	<i>Vib. cholera.</i>
phenolate	7 ^d - 9 ^d	5 ^d - 6 ^d	6 ^d - 7 ^d	9 ^h + 12 ^h -
o-cresolate	7 - 9	5 - 6	6 - 7	9 + 12 -
guaiacolate	13 - 15	7 - 9	10 - 12	24 + 36 -
thymolate	6 ^h ± 9 ^h -	2 ^h + 3 ^h -	3 ^h + 6 ^h -	5 ^m ± 10 ^m -
picrate	13 ^d - 15 ^d	8 ^d - 10 ^d	12 ^d - 15 ^d	24 ^h + 36 ^h -
pyrocatechinate	10 - 12	8 - 10	10 - 13	12 + 18 -
resorcinate	15 - 18	10 - 12	12 - 15	24 + 36 -
hydroquinonate	5 + 6 -	2 + 3	4 + 5 -	2 + 3 -
pyrogallate	10 - 12	6 - 8	8 - 10	12 + 18 -
phloroglucinate	30 - 35	20 - 25	25 - 30	36 + 48 -
Control	8 ^d ±	5 ^d ±	6 ^d ±	18 ^h ±

The results with Ca salts and NH₄ salts are nearly the same as with Na salts and they need not be recorded here.

IV. STERILIZING ACTION OF PHENOLS AT THE SAME pH.

The sterilizing action of phenols was tested at the same pH in this experiment. In reference to the sterilizing action at a high pH, I record the results with thymol.

Results obtained shown in the following table.

TABLE 4. Sterilizing action of phenols at the same pH.

Phenols	Conc.	pH	Surviving period			
			<i>Staph. pyogen.</i>	<i>Prot. vulgar.</i>	<i>Bac. typhos.</i>	<i>Vib. cholera.</i>
Phenol	N/1000	5.45	7 ^d + 8 ^d -	4 ^d + 5 ^d -	5 ^d + 6 ^d	9 ^h ± 12 ^h -
Picric acid	N/200000	"	4 + 5 -	3 ± 4 -	3 + 4 -	3 + 6 -
o-Cresol	N/2000	"	8 + 9 -	5 ^d + 6 ^d -	6 + 7 -	9 + 12 -
Pyrocatechin	N/4000	5.64	2 + 3 -	24 ^h + 36 ^h -	2 ^d ± 3 ^d -	30 ^m + 45 ^m -
Resorcine	N/1500	"	14 + 15 -	9 ^d ± 10 ^d -	12 ± 13 -	24 ^h + 36 ^h -
Hydroquinone	N/1000	"	12 ^h + 18 ^h -	6 ^h + 9 ^h -	9 ^h ± 12 ^h -	2.5 ^m ± 5 ^m -
Pyrogallie acid	N/150000	5.71	15 ^d - 18 ^d	7 ^d - 10 ^d	12 ^d - 15 ^d	12 ^h + 18 ^h -
Phloroglucine	N/1000	"	20 - 25	10 - 15	15 - 20	24 + 36 -
Thymol	N/1000	6.13	2 ^h + 3 ^h -	60 ^m ± 90 ^m	90 ^m + 2 ^h -	1 ^m ± 2.5 ^m -
Control			8 ^d	5 ^d ±	6 ^d	18 ^h ±

V. SUMMARY.

The results noted in tables 2~3, and 4, concerning the sterilizing action of phenols and their salts, may be summarised as follows:

(1). At the same concentration, the sterilizing action of picric acid is the strongest among 10 phenols, and next to this is thymol. The sterilizing action of hydroquinone is slightly weaker than thymol, and pyrocatechin and then pyrogalllic acid come next to thymol.

(2). Sterilizing action of phenol is strong only at $N/10$, and at concentration lower than $N/100$ it has no sterilizing power.

(3). The sterilizing action of guaiacol and resorcine is very feeble and rather seems to have no power except for *Vib. cholerae*. At $N/1000$ they have rather evidently promoting power for bacterial life.

Phloroglucine has totally no bactericidal action, but has rather evidently promoting action on bacterial life.

(4). Salts of hydroquinone have relatively strong sterilizing action, which is more evident with salts of thymol.

Salts of phenols other than thymol and hydroquinone, have no bactericidal action, and show rather promoting action on the bacterial life.

From these facts we see that anions of thymol and hydroquinone have bactericidal action.

(5). The strong sterilizing action of picric acid is chiefly due to the low pH of picric acid in adding to the poisoning action of molecular state of picric acid.

The strong sterilizing action of thymol is chiefly due to the poisoning action of molecular state of thymol and partly due to the action of thymol anion.

(6). The relative strength of the sterilizing action of *o*, *m*, and *p* isomers such as pyrocatechin (*o*) and resorcine (*m*), and hydroquinone (*p*), and also pyrogalllic acid (*o*) and phloroglucine (*m*), are as follows:

$$m < o < p.$$

The cause of these differences is due to the difference of chemical constitution of each isomer.

(7). The relation between the strength of the sterilizing action of phenols and the number of OH group which give the acid character to phenols may be expressed as follows:



Biochemical Investigation of Mosaic Disease of Tobacco Plants. VI.

On Ascorbic Acid Oxydase and Saccharase in the Leaves of Healthy and Mosaic Plants.

(pp.306 ~310)

By Y. OKUDA, K. KATAI and E. MURATA.

(Agricultural Chemical Laboratory, Kyushu Imperial University; Received March 11, 1940.)

Über die Verwitterung der Eruptivgesteine. VI

Über den Verwitterungskomplex.

(SS. 311~320)

Von Mituru HARADA.

(Landwirtschaftliche Hochschule Tottori; Eingegangen am 22. 3. 1940.)

Der $\text{SiO}_2\text{-Al}_2\text{O}_3$ -Niederschlag wurde wie folgt hergestellt. 1 N. AlCl_3 -Lösung wurde mit Na-Silikatlösung bekannten Gehalten titriert, bis sich der Niederschlag abgesetzt und die darüber stehende Flüssigkeit klar geworden ist. Zwecks Gewinnung von Niederschlägen mit niedrigem Verhältnis $\text{SiO}_2\text{:Al}_2\text{O}_3$ wurde eine Lösung gebraucht, die Na-Silikat und NaOH enthält. Der Niederschlag wurde filtriert und mit 95%igem Alkohol Cl-frei gelassen, alsdann der Alkohol durch Einblasen von Luft verjagt. Der $\text{SiO}_2\text{-Fe}_2\text{O}_3$ -Niederschlag wurde in gleicher Weise hergestellt.

Beim $\text{SiO}_2\text{-Al}_2\text{O}_3$ -Niederschlag ist die Löslichkeit der Kieselsäure in der Oxalsäure-Kaliumoxalatlösung (18.4 g $\text{K}_2\text{C}_2\text{O}_4\cdot\text{H}_2\text{O}$, 3.2 g $\text{H}_2\text{C}_2\text{O}_4\cdot 2\text{H}_2\text{O}$ im Liter) um so größer, je kleiner das Mol.-Verhältnis $\text{SiO}_2\text{:Al}_2\text{O}_3$ ist. Niederschläge, deren Mol.-Verhältnis kleiner als 2 ist, sind fast ganz löslich, dagegen ist beim Niederschlag mit dem Verhältnis 6 nur 11% der Kieselsäure löslich. Die Tonerde wurde zu 87% beim Mol.-Verhältnis 6, zu 99~100% beim Verhältnis 0,8~2,3 durch die Oxalsäure-Kaliumoxalatlösung gelöst. Beim $\text{SiO}_2\text{-Fe}_2\text{O}_3$ -Niederschlag ist das Eisen fast ganz, die Kieselsäure aber nur 28~58% in der Oxalsäure-Kaliumoxalatlösung löslich.

Durch wiederholtes Trocknen und Anfeuchten wurde die Kieselsäure im $\text{SiO}_2\text{-Al}_2\text{O}_3$ -Niederschlag mit einem Mol.-Verhältnis $\text{SiO}_2\text{:Al}_2\text{O}_3$ größer als 2 schwer löslich, dagegen wurde diese Löslichkeit bei Mol.-Verhältnis kleiner als 2 nicht verändert; die Löslichkeit der Tonerde wurde bei Mol.-Verhältnis größer als 6 vermindert. Durch dieselbe Behandlung im Dunkeln wurde die Löslichkeit der Kieselsäure im $\text{SiO}_2\text{-Fe}_2\text{O}_3$ -Niederschlag allgemein stark Verminderung der Löslichkeit des Eisens sehr gering. In dem in Oxalsäure-Kaliumoxalatlösung löslichen Teil ist das $\text{SiO}_2\text{/Al}_2\text{O}_3$ Verhältnis kleiner als 2 (0,6~2,0) und das $\text{SiO}_2\text{/Fe}_2\text{O}_3$ Verhältnis ist etwa 1 (0,6~1,1). Während das nicht getrocknete frische Aluminium-

hydroxyd in der Oxalsäure-Kaliumoxalatlösung löslich ist, wird es durch wiederholtes Austrocknen und Befeuchten unlöslich. Eisenhydroxyd löst sich in dieser Oxalatlösung im Dunkeln auf, und die Veränderung der Löslichkeit nach wiederholtem Trocknen und Befeuchten ist sehr gering.

Aus den obigen Ergebnissen wird erhellt, daß die Tonerde im $\text{SiO}_2\text{-Al}_2\text{O}_3$ -Niederschlag und Eisen im $\text{SiO}_2\text{-Fe}_2\text{O}_3$ -Niederschlag nur schwache Alterungsvorgänge zeigen und wie das frisch gefällte Hydroxyd chemisch reagiert, während die Kieselsäure schnell altert.

Der in Böden gebildete Verwitterungskomplex besteht aus 3 Fraktionen, nämlich aus einem Komplex (A_1), löslich in der Oxalsäure-Kaliumoxalatlösung im Dunkeln, einem Komplex (A_2), zersetzbar in heißer konzentrierter Salzsäure, aber unlöslich in dieser Oxalatlösung, und einem Komplex (B), nur zersetzbar in heißer konzentrierter Schwefelsäure.

Der Komplex A_1 im Boden wird folgendermaßen bestimmt. Man wägt 0,5~1 g des zerkleinerten Bodens in einer Stohmanschen Halbliterflasche, übergießt den Boden mit 250 cm der Oxalsäure-Kaliumoxalatlösung, schüttelt im Dunkeln 1/2 Stunde lang aus und filtriert, alsdann bestimmt man SiO_2 und Al_2O_3 in der Lösung. Das gelöste Fe_2O_3 ist fast ganz im freien Zustande.

Freies Eisenoxyd (E) (s. Mitteilung IV) und die freie Tonerde (T), die in heißer 10%iger Na_2CO_3 -Lösung löslich ist, werden bestimmt.

Komplex A_2 =(das in konz. Salzsäure zersetzbare SiO_2 , Al_2O_3
und Fe_2O_3)- A_1 - E - T

SiO_2 , Al_2O_3 und Fe_2O_3 im Komplex A_1 , A_2 und B in verschiedenen Bodenarten auf Eruptivgesteinen wurden bestimmt. Vulkanische Aschenböden enthalten Komplex A_1 , A_2 und B. Die Böden aus Hornblende-Andesit sind reich an Komplex A_1 und A_2 , Granit-, Diorit-, Quarztrachyt-, Augit-Andesit- und Basalt-Böden enthalten Komplex A_2 und B. Die Böden aus lockerem vulkanischen Lapilli enthalten große Mengen von Komplex A_1 . Quarzkeratophyrboden ist reich an Komplex A_2 .

Der Verf. hat gefunden, daß der Humus im Boden in Gegenwart von Eisenhydroxyd durch 3%ige H_2O_2 -Lösung oxydiert wird, wobei ein Teil des Aluminium in Komplex A_1 als Oxalat in Lösung geht, und ferner durch die folgende Behandlung das ganze Aluminium in Komplex A_1 aber kein gealtertes Aluminiumhydroxyd gelöst wird. 0,5~1 g Boden, 0,5 g Hydrochinon, 0,05 g Eisenhydroxyd und 60 cm 3%ige H_2O_2 -Lösung wird in ein Becherglas gebracht, mit einem Uhrglase bedeckt und auf siedendem Wasserbade erhitzt. Beim Steigen der Temperatur bis zu 70~80° reagiert das Gemisch energisch. Ist die Zersetzung des H_2O_2 beendet, wird nach Zusatz von 1 g NH_4Cl filtriert und das gelöste Aluminium bestimmt.

Studies on Acetone-Butylalcohol Fermentation. (III).

Utilization of various protein-rich raw materials as
N-source for acetone-butylalcohol fermentation.

(pp. 321~330)

By Sigeyosi HORIE.

(Agricultural Chemical Laboratory, Kyusyu Imperial University, Fukuoka ;

Received March 25, 1940.)

On the Colorimetric Determination of Vitamin B₁.

(pp. 331~339)

By Yosito SAKURAI, Tyoten INAGAKI and Sizu OMORI.

(Research Laboratory of Meiji Sugar Co. ; Received March 22, 1940.)

The procedure described by Prebluda and Melnick which involves the use of diazotized *p*-aminoacetophenone as the reagent for the determination of vitamin B₁ is modified to the following simpler method, concentrating the vitamin B₁ in the extract by the adsorption on acid clay.

The sample is extracted with water or dilute alcohol at pH 4.5. An aliquot portion of the extract is adsorbed with 0.2 g refined acid clay for about ten minutes, and then centrifuged. To the centrifuged adsorbate 3 cc of water, 3 cc of alcohol containing phenol and 6 cc of freshly prepared reagent are added. The reaction is complete in 1 hour, after which 8 cc of alcohol and 5 cc of xylene are added followed by vigorous stirring for 2 minutes. On standing the xylene layer separates out easily showing pink color. After clarification by centrifugation the xylene layer is taken in 1 cm cuvette and the extinction is measured by Pulfrich's photometer using S 53 filter (pale green).

The extinction coefficient of 30 micrograms of vitamin B₁ hydrochloride is as follows.

	Without acid clay	With 0.2 g acid clay
Average	0.323 (average of 16 measurements)	0.280 (average of 12 measurements)
Maximum	0.337	0.299
Minimum	0.302	0.267

Über die Synthese von α -Naphthylelessigsäure, β -Indolylbuttersäure und β -Indolylpropionsäure.

(SS. 340~344)

Von Kinjiro TAMARI.

(Landwirtschaftliches Chemisches Laboratorium der Kaiserl. Universität, Tokyo;

Eingegangen am 26. 3. 1940.)